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DEPARTMENT OF BIOCHEMISTRY

BERKELEY, CALIFORNIA 94720

29 June 1988

Dr. Michael Marron
Office of the chief of Naval Research
Arlington, VA 22217-5000

Dear Dr. Marron:

With this letter, I am submitting the FINAL REPORT for ONR Contract N00014-85-K-0363 for the project titled "Molecular Basis of Cell-Cell Recognition in Yeast". The research carried out under this contract has resulted in a significant increase in our understanding of the structures of the "recognizer" macromolecules and their "sites of recognition", which are also large glycoproteins. As suggested in our original application, this study provides insight into the mechanisms for cell recognition in systems more closely related to the fouling of naval ships such as occurs from adhesion by marine organisms.

Sincerely,

C. E. Ballou

C. E. Ballou
Professor of Biochemistry

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION U	1b RESTRICTIVE MARKINGS NA
2a SECURITY CLASSIFICATION AUTHORITY NA	3 DISTRIBUTION / AVAILABILITY OF REPORT Unlimited
2b DECLASSIFICATION / DOWNGRADING SCHEDULE NA	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) NA	5 MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a NAME OF PERFORMING ORGANIZATION University of California 6b OFFICE SYMBOL (If applicable) NA	7a NAME OF MONITORING ORGANIZATION Office of Naval Research
6c ADDRESS (City, State, and ZIP Code) Department of Biochemistry Berkeley, CA 94720	7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000
8a NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research 8b OFFICE SYMBOL (If applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-85-K-0363
8c ADDRESS (City, State, and ZIP Code) 800 N Quincy Street Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 61153N PROJECT NO: RR04106 TASK NO: WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)

Molecular basis of cell-cell recognition in yeast

12 PERSONAL AUTHOR(S)
Clinton E. Ballou

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final	FROM 7/1/85 TO 6/30/88		

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	yeast cell wall; Saccharomyces kouyveri; sexual agglutination, mannoprotein; proteolysis; (KD)
08			

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The heat-labile sexual agglutinin from Saccharomyces kluyveri 17-cells was isolated and shown to consist of 3 domains, a glycopeptide part containing all of the N-linked oligosaccharides and making up one-third of the protein that was embedded in the cell wall and held the agglutinin on the cell surface, a carbohydrate-free polypeptide portion of 60 kDa that possessed the recognizer site, and a glycopeptide with O-linked carbohydrate that interconnected the other 2 domains. The heat-stable sexual agglutinin from S. kluyveri cells was isolated and shown to consist of a large glycopeptide making up 80% of the mass of the agglutinin that was anchored in the cell wall and a small glycopeptide of 25 kDa that had the site of recognition that interacted with the 60 kDa 17-cell fragment. The 25 kDa site was released from the intact molecule by reducing agents, which shows that it is attached by a disulfide bond, and partial sequence data and smaller proteolytic fragments have been obtained. These results demonstrate that the sexual agglutination reaction in yeast has many similarities to the fertilization reaction in higher eucaryotes.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	U
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. E. Schmell or M. Marron	22b TELEPHONE (Include Area Code) (202) 696-4760
	22c OFFICE SYMBOL ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

88 7

DEPARTMENT OF THE NAVY
Office of Naval Research

FINAL REPORT ON CONTRACT N00014-85-K-0363 DATE:

PRINCIPAL INVESTIGATOR: Dr. Clinton E. Ballou
Department of Biochemistry
University of California
Berkeley, CA 94720

CONTRACTOR: University of California

CONTRACT TITLE: Molecular Basis of Cell-Cell Recognition in Yeast

START DATE: 1 July 1985

TERMINATION DATE: 30 June 1988

RESEARCH OBJECTIVES: To isolate and characterize the sexual agglutination factors from the two haploid cell types of the yeast Saccharomyces kluyveri, with the eventual goal of defining the precise molecular structures of the two agglutinins, thereby contributing to a better understanding of all types of cellular adhesion.

PROGRESS: During year 1 of this project, evidence was obtained that, during glucanase digestion of S. kluyveri cells to solubilize the agglutinins, extensive proteolysis of the factors occurred. This could be prevented by purifying the glucanase until it was protease-free, and with this glucanase it became clear that the intact agglutinin from S. kluyveri 17-cells (α-agglutinin) was larger than 200 kDa. During year 2 of this project, the α-agglutinin was isolated in intact form from wild-type cells and from a glycosylation-defective mutant that makes the agglutinin with a reduced carbohydrate content. The wild-type and mutant agglutinins are polydisperse, the first having an apparent Mr >400,000, while the second is somewhat smaller. Both agglutinins are converted by controlled proteolysis sequentially to smaller active fragments of 150 and 60 kDa. The 60 kDa fragment was purified by HPLC and found to contain only O-linked carbohydrate, which demonstrated that the N-linked oligosaccharides are restricted to that part of the agglutinin molecule that anchors the macromolecule in the cell wall. For the last year of this project, we have concentrated on purification and characterization of the 16-cell a-agglutinin. The active recognition site of this agglutinin was released from whole cells by treatment with dithiothreitol, after which it was purified by gel filtration and HPLC. The fragment is a glycopeptide with Mr 25,000 and it travels during gel electrophoresis as a mixture of closely related bands, all of which can be stained by exposing the gel to ^{125}I-α-agglutinin, which recognizes and binds to the glycopeptides. A partial amino acid sequence has been obtained for the a-cell recognition site, and we are presently purifying fragments produced by treating this glycopeptide with selected proteases.

PUBLICATIONS AND REPORTS

1. Cell-cell recognition in yeast. Molecular nature of the sexual agglutinin from Saccharomyces kluyveri 17-cells. K. Weinstock and C. E. Ballou (1986) J. Biol. Chem. 261, 16174-16179.

PUBLICATIONS AND REPORTS (CONTINUED)

2. Cell-cell recognition in yeast. Isolation of intact α-agglutinin from Saccharomyces kluyveri. R. D. Lasky and C. E. Ballou (1988) Proc. Natl. Acad. Sci. USA 85, 349-353.

3. Tunicamycin inhibition of epispore formation in Saccharomyces cerevisiae. K. G. Weinstock and C. E. Ballou (1987) J. Bacteriol. 169, 4384-4387.

4. Characterization of the a-agglutinin from Saccharomyces kluyveri a-cells. M. S. Lewis and C. E. Ballou (1988). In preparation.

TRAINING ACTIVITIES

Keith Weinstock -- Completed work for Ph.D. while supported under this project. Presently employed as a postdoctoral researcher at the Laboratory of Eucaryotic Gene Expression, Frederick Cancer Research FAcility, Frederick, MD 21701.

Richard Lasky -- Conducted postdoctoral research while supported under this project. presently employed on the research staff of Imreg, Inc., Cambridge, MA.

Mark Lewis -- Presently supported and working on this project. Expected to complete his doctoral thesis by the end of this year.

Grant Kalinowski -- Undergraduate student in biochemistry, supported on this project during the summer of 1987 and trained in basis laboratory work.